The Ohio Naturalist,

PUBLISHED BY

The Biological Club of the Ohio State University.

Volume VI. MAY, 1906. No. 7.

TABLE OF CONTENTS.

A REDUCING DIVISION IN ASCARIS.

ROBERT F. GRIGGS.

For a long time, comparatively speaking, Ascaris has stood in the way of a consistent rational interpretation of the phenomena of reduction. It has been regarded as the one case in which the divisions of oogenesis and spermatogenesis were *proven* to be equational and not qualitative. The growing appreciation of the bearing of Mendel's Law and the course of recent work on reduction have served to make it more and more of a stumbling block in the way of those who tried to understand the mechanism of heredity. So great is our faith in the uniformity of nature that it was impossible to believe that Ascaris was unique. Either the theories of reduction built on the observed facts must be insufficient or there is something yet unobserved in this form which would bring it into harmony with the law.

It was for this reason that Tretjakof (8) undertook the problem and published simultaneously two papers on the Oogenesis and spermatogenesis of Ascaris. His conclusions were similar to those reached in the present study and apparently he saw many of the same sorts of figures as has the present writer, but his drawings are not conclusive and as Gregoire (3) says,* simply open the question anew. The same may be said of the work of Moszkowski (7) whose paper is unillustrated except for four text figures.

It is, then, a matter of considerable satisfaction to be able to present what seems to the writer conclusive evidence that the reduction division of Ascaris is a true Reducing Division in Weisman's sense.

* * * " de plus il faut avouer que les figures de Tretjakof, sont fort difficiles à élucider et qu'il est malaisé de se faire une opinion d'après leur inspection.

" Les recherches de Tretjakof montrent que l'Ascaris n'etait pas encore completement etudié, mais elles ne me semblent pas elucider definitivement cet ojet difficile."

In carrying forward the work I have been greatly aided by two men who have "seen me through" it, and checked up and verified my observations as the work proceeded. They are Professor Francis L. Landacre of the department of Zoology and Professor John H. Schaffner of the department of Botany, of the Ohio State University. To both I wish to extend my heartiest thanks. The slides on which the work was done belong to Professor Landacre and have been used by the students in the Department of Zoology, studying principally the later stages, for several years. They are cut from a female of the variety *bivalens* and are arranged in series lettered backward from A which contains two-and four-celled embryos, to M in which are found the early stages but little removed from the resting nuclei of the oogonia. All of the nuclei drawn for the plate of this paper except fig. 12, were found in series M and L.

The difficulties in Ascaris are, I am inclined to believe of two sorts: first the problem of staining and second, the minuteness of the critical stages. The slides are stained with Heidenhain's Iron Alum Heamatoxylon. To find material in which the nuclei were properly stained it was necessary to select extremely faint slides in which all of the stain had been drawn from the cytoplasm and spindle leaving the nuclei standing out clearly by themselves. Even then the spirems are often so closely knotted together and deeply stained as to make resoulution impossible. In the matter of magnification I find that the 1–12 objectives which have been mostly used are far inferior for this work to the 1–16 which was used with a variety of oculars. Of these the 1–2 inch was the most saitsfactory. Lower oculars do not cut the plane of focus sharp enough to enable one to follow out the spirems.

The variety bivalens is a more favorable object for study than the variety univalens, for as Tretjakof remarks, the development of the two tetrads usually proceeds unequally so that one is often found in a much more advanced stage than the other. This frequently enables one to understand figures which without such aid would be difficlut of interpretation. In fig. 9, for example, one tetrad is clearly differentiated while the loop that will form the second is still much twisted. This might be interpreted in a number of ways were it not for its fellow which requires us to homologize the loop to a tetrad.

The process of tertad formation in Ascaris is in close agreement with that more recently described in "many Arthropods, Amphibia" (Montgomery (6) and the higher plants, though the appearances are quite different in the different cases. In all these there is a precocious longitudinal division of the spirem, which through subsequent contraction becomes more or less invisible. Contemporary with this or following it is a conjugation of the chromosomes (already longitudinally divided) two by two forming combinations of four members.

From the resting nucleus a continuous spirem is formed in the usual manner (figs. 1–3). In Ascaris the spriem is very closely wrapped so that it is a very difficult object to resolve and cannot be untangled and traced out with confidence. This however is a matter of no great moment in the present discussion since the occurrence of such a spirem is well known. In some cases (fig. 1–2) even before the spirem is formed the chromatin is distinctly separable into two masses. Such a separation may (fig. 5) or may not (fig. 3–4) be evident in the spirem.

Before the contraction of the spirem has proceeded far the granules which are strung along the linin thread become doubled (fig. 4). At first very difficult to observe, the distance between the doubled granules becomes greater and greater till the linin thread splits and two parallel sprimes are formed (fig. 5). It has been maintained recently by Berghs (1) that in the flowering plants these doubled granules arise not by a longitudinal splitting of the spirem as has hitherto been supposed but by the conjugation of the granules from two separate strands of linin. Whether the doubled spirem in Ascaris arises in such a manner or by a split does not seem to me susceptible of complete demonstration. I see, however, no reason for abandoning the older interpretation; while there are several indications that point toward a splitting rather than to a conjugation. (1) The spirem at the earliest stage where the doubling appears (i. e. earliest as judged by the relative state of contraction of the spirem) is of approximately the same length (fig. 4) as the single spirem preceding (fig. 3), while if a conjugation took place it would be of only half the length. (2) At the earliest stage where the doubling is visible, judged by the same criterion, the granules lie exceedingly close together and from this stage they *recede* up to a stage represented by fig. 5. while if they were the result of conjugation we should expect the opposite.

Very frequently in split spirems (fig. 5) the linin at one end is bent into the form of a square with a prominent granule at each corner. The granules are so much more prominent than the linin thread upon which they are strung that one might easily suppose that he was looking at the *end* of a set of granules doubly split instead of at the *side* of a continuous spirem. In the cases observed however there was no great difficulty in tracing the course of the spirem and showing that such was only a superficial appearance. The spirem in this stage often foreshadows quite plainly the tetrads destined to be formed from it. In its contraction it is often thrown into two loops each arm of which is double giving therefore two groups of four strands each.

The contraction of the spirem continues and is manifested not only by a shortening in length but also by a drawing together of the sprit glanules. This usually goes so far that the longitudinal split becomes very difficult or impossible to observe (figs. 6–8). The two loops destined to become the tertads now become more and more definite and begin to break apart (fig. 6) sometimes becoming twisted (fig. 7) in a way that is inconceivable were they due to a longitudinal split of the original thread. Sometimes (fig. 8) the two arms of a loop are of different lengths and this again seems to me fatal to any interpretation of their origin by a longitudinal split. In this stage the longitudinal split becomes visible again by the moving apart of the two strands so that we see anew the four chromatids which form the tetrad (figs. 8–9). So marked a contraction as is shown in figs. 7 and 8 is not universal. Sometimes the four strands are visible through the whole process. Fig. 10 represents a stage but little later than fig. 6, where the spirem has not yet broken as in that case though the granules are more closely packed together to form the four elements in each of the tertads; but the approximation of the two sides of the loop is still incomplete in the upper tetrad. The chromosomes now become more compact and gradually take their position in the tetrads (fig. 11.) All traces of the linin thread may have disappeared by this time or the original linin may persist between the two tetrads as in fig. 11 and by the attachment of its split ends show plainly which chromosomes are the result of conjugation and which of splitting. From a stage represented by fig. 11 it is an easy step to the mature tetrad ready for the first division (fig. 12). The only change consists in a further shortening of the chromatids.

In the process just described the ends of the loops which form the tetrads are connected by two double linin threads which twist or pass close together at a common point, corresponding to the bases of the two original loops. Because of their being thus drawn together the resultant tetrads nearly always stand at an angle to each other instead of extending in the same straight line, see especially figs. 6 and 7. This angle persists until just before the separation of the dyads in the first mitosis (fig. 12) and is very noticeable. While it cannot be regarded as positive evidence either way, it is not easy to explain such an angle on any assumption of double longitudinal splitting but it corresponds with and helps to corroborate the looping shown to take place in tetrad formation.

After the tetrads are well formed the facts of the process of reduction are so well known as to require no amplification here. From each tetrad by the two maturation divisions are passed out successively a dyad and a monad, leaving one monad from each of the tetrads to form the resulting female producleus. Since the tetrads arise by a conjugation of two longitudinally divided chromosomes, one of these maturation divisions is transverse and qualitive representing a true Reducing Division in Weisman's sense. There next arises the question as to which of the two divisions is the Reducing Division. This point the writer does not hesitate to say is very difficult, or perhaps incapable of complete demonstration in Ascaris. It has seemed to him that the presence of the Reducing Division was the all important fact and that the matter of deciding which division was qualitative was of much less importance. Because of this and because of the great difficulty of the matter I have not tried seriously to determine the question in the present investigation. The different chromatids in the tetrads are so similar and so difficult to find in favorable positions where all four of them can be seen at once that it is only with great reserve that statements as to the identity and origin of the separate dyads of the first division can be made. But in this matter the angle between the tetrads may give a clue, not, however in my judgment amounting to proof. By inspecting such stages a figs. 9 and 10 it will be seen that if we take the nearest common plane of the two tetrads, that in which the angle between them would lie, were it a plane angle (the plane of the paper in the cases cited) then we find that the equivalent chromatids arising by a split, lie perpendicular to that plane and the dissimilar chromatids arising by a transverse break lie parallel to that plane. Applying this to fig. 12 in which the tetrads are oriented for the first division but not yet drawn out of their original angle we find that the first division would be the qualitative for it is the dissimilar dyads which lie on the different sides of the eauatorial plate of the spindle and will pass to the different cells in mitosis.

The results given above were arrived at after examination of many hundreds of nuclei in the critical stages. The ones selected for the figures are unusual only in their clearness and in the favorable position of the parts. Of all the nuclei seen about half were so strongly contracted as to be impossible of resolution. Of the others all but two or three were clearly interpretable as stages in the process outlined above. A few, about 1–3 per cent. should be interpreted either as products of folding or of a double longitudinal division. None were found which could be interpreted as products of the latter process which did not lend themselves equally well to the other interpretation.

Inasmuch as the results of the present investigation are diametrically opposed to those reached by Brauer (2) on the spermatogenesis of the same object, it might seem difficult to bring the observations of Brauer into harmony with those of the present writer. But such is not at all the case. One point which Brauer lays great stress upon and which is at first sight most convincing, is that the granules are sometimes clearly doubly split at a very early period. Whatever the significance of this grouping of granules in fours may be, it is not necessarily a precourser of the reduction division. Such groups of four granules as Brauer shows (fig. 22–24) are frequently very abundant in the nuclei of the wall cells of the uterus which are not, of course, in preparation for a reduction division. He has several figures (35, 37, 41, 42) in which the doubled loops shown in my fig. 6 are very plain. He does not, however, follow the gradual approximation of the sides of the loops but supposes them to straighten out into a single semicircular band which by transverse division forms the two tetrads. During all these stages he supposes that the spirem is composed of the doubly split granules of the early prophases, believing, doubtless, that his inability to see them was due to the very unfavorable positions which such objects would inevitably assume. He does, however, show in small portions of figures 34, 36 and 41, places where the spirem is represented as composed of three or four strands instead of two which the present writer has invariably found. Beyond these points there is no greater difference in our observations than is probably due to differences in the sex cells of male and female animals.

Montgomery (6) has pointed out that the tetrads are of unequal size. My own studies have not been carried carefully into the maturation mitoses where Montgomery made his observations but what I have seen of these stages tends to confirm his statements. The earlier stages also offer strong confirmatory evidence of their truth. As has been mentioned one of the tetrads is almost always slower in its formation than the other, being derived apparently from longer more contorted segments of the spirem thread. His contention is that there is always a conjugation of *similar* chromosomes to form a tetrad. This would seem to be correct in the main but it appears to be not without exceptions, see fig. 8.

SUMMARY.

The foregoing observations seem to show conclusively that the tetrads in the eggs of *Ascaris megalocephala bivalens* arise not by a double longitudinal split of the original spirem thread but by a folding of adjacent segments together (conjugation of univalent chromosomes) together with what is believed to be a single longitudinal split. The two split loops which form the two tetrads appear very early in the contuinous spirem and in their later development simply break apart, shorten, thicken, and straighten out till the tetrads are formed.

Since of each tetrad only one component chromosome remains in the ripe ovum, there is a Reducing Division in Wesimann's sense by which paired chromosomes are separated from each other in the egg and the hereditary characters transmitted by the chromosomes, thereby modified.

Literature Cited.

With two exceptions, I have listed only the papers directly referred to in the text. The exceptions are Guyer's extremely important work published before the rennaissace of Mendel's Law, which have not received from writers on kindred subjects, the attention they deserve.

1. Berghs, Jules. La Formation des Chromosomes Heterotypiques dans la Sporogenese Vegetale. II Depuis la Sporogonie jusqu'an Spireme Definitiv. La Cellule **21** : 383–397 Pl. 1. 1904.
2. Brauer, A. Zur Kenntniss der Spermatogenese von Ascaris megalocephala. Archiv f Mik. Anat. u. Entw. **42** : 168. 1893.
3. Gregoire, Victor. Les Resultats Acquis sur les Cineses de Maturation. Premiere Memoire: Revue Critique de la Literature. La Cellule **22** : 221–376. 1905. Material cited at p. 330.
4. Guyer, M. F. Spermatogenesis of Normal and Hybrid Pigeons. Chicago, 1900.
5. ————. Hybridism and the Germ-Cell. Univ. Cincinnati. Bull. 21. 1902.
6. Montgomery, Thos. H. Some Observations and Considerations on the Maturation Phenomena of Germ Cells. Biol. Bull. **6** : 137. 1903.
7. Moszkowski, Max. Zur Richtungskorperbildung von Ascaris megalocephala. Archiv f. Mik. Anat. u. Entw. **59** : 388. 4 fig. 1902.
8. Tretjakof, D. Die Bildung der Richtungskorperchen in den Eieren von Ascaris megalocephala. Archiv. f. Mik. Anat. u. Entw. **65** : 358–583. Pl 21. Die Spermatogenese bei Ascaris megalocephala. p. 383–435. Pl. 22, 23. fig. 1. 1905.

Explanation of Plate XXXIII.

The figures were drawn with a Leitz 1–16 oil immertion objective and a Bausch and Lomb 1–2 in. ocualr. They were drawn with the aid of a camera lucida and are reproduced 2–3 their original size.

Fig. 1. A nucleus just passing from the resting stage. The nuclear membrane is *extremely* faint, being on the verge of disappearance.

Fig. 2. The chromatin network well broken up and on the way to the formation of the continuous spirem.

Fig. 3. A continuous spirem. This is wrapped so tightly that it is not possible to be certain all the strands run exactly as drawn. The nucleus was, however, in this stage and about as drawn.

Fig. 4. Slightly later than the last, showing the first appearance of doubled granules on the still single linin spirem. As in fig. 3, the spirem is too complicated to be followed with certainty but it in the main as represented.

Fig. 5. A continuous spirem singly split. The spirem may be traced by focusing from the granules at the right around the elbow above and back on the left side where after crossing twice it turns and passes under the elbow to the granule next the starting point. In addition to this there can be traced from the first granule, a loop passing under the other large granules where its relations cannot be made out. It is not impossible that this loop is not linin at all but some cytoplasmic condensation. It is not like the rest of the spirem in appearance. On superficial examination the right portion might be mistaken for an end view of a tetrad with bridges between the rods but its relations to the whole clearly negate any such possibility.

Fig. 6. A split spirem doubled on itself to form the two tetrads. It has already broken apart at the upper end of the right tetrad leaving two loose ends, connected by a faint strand of dense sytoplasm.

Fig. 7. Spirem in which one strand is twisted entirely around the other in a manner impossible in a split rod. The two ends of the loops are beginning to break apart or perhaps have already broken but remain in close contact.

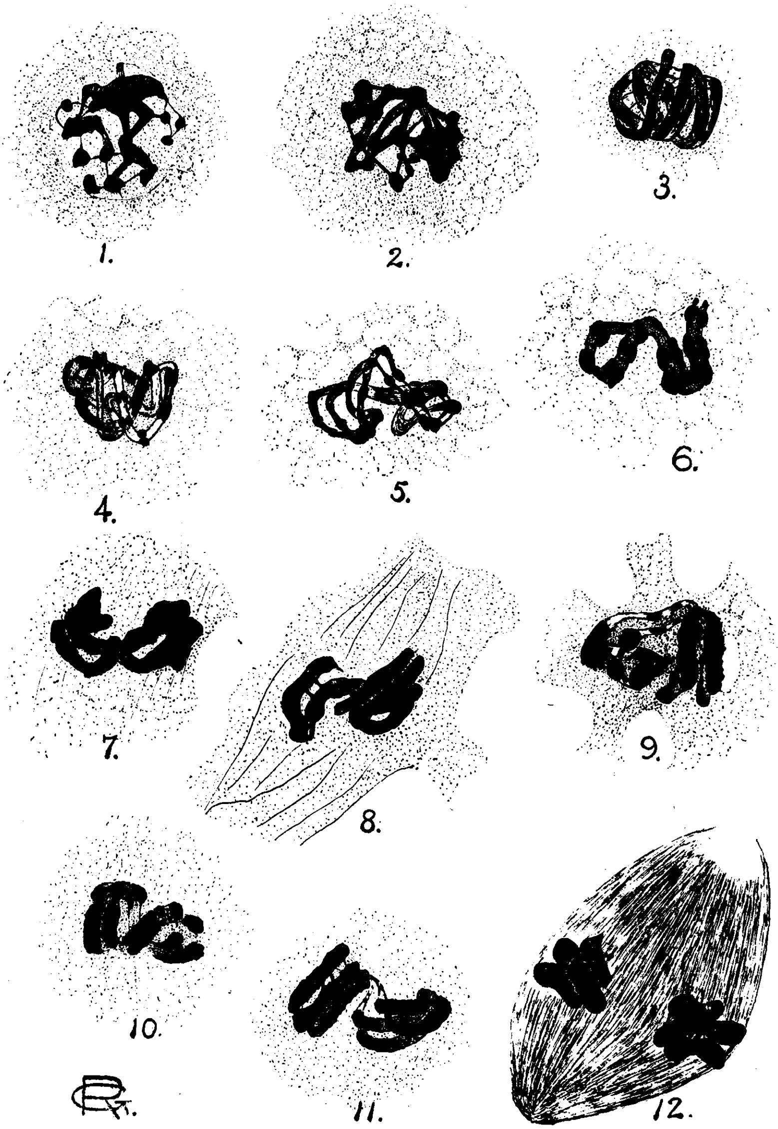
Fig. 8. One tetrad nearly formed, the other lagging. The relations of the four rods to the right are not possible to make out precisely. On the left the loop of the original spirem is still evident. This shows the longitudinal split faintly in the distal end. Such a figure as this might easily be interpreted as due to a double split. The loop looks at first sight like the incompletely separated ends of a longitudinal split. But at the point of junction the distal (left) arm turns up and then bends down to meet the other which in like manner turns down and then up making a rounded loop perpendicular to the plane of the paper. One arm of the loop is also much shorter than the other but does not seem to be cut off or disturbed.

Fig. 9. A nucleus in which one of the two tetrads is much more completely formed than the other. The right tetrad is seen to be composed of four rods two above the others. The spirem has completely broken across between the two arms of the loop and in one side the longitudinal split is also complete while in the other there remains a bridge across between the two portions. At the base of this tetrad both arms are seen to be continuous with the spirem which starting from one arm bends around and is twisted on itself in the position of the left tetrad, returning to the second arm of the right tetrad. In the parallel strands near the right tetrad are seen two pairs of small grauules which may be chromatin or merely thickenings of the linin thread. Were it not for the evidence of the rest of the loop these might be taken to have arisen by a longitudinal split but such an interpretation is clearly impossible of the twisted spirem of which they are a part.

Fig. 10. A continuous split spirem of almost the same age as figure 6, in which the tetrads are clearly forshadowed though not yet differentiated. Contraction with consequent obliteration of the chromatin granules has gone further than in fig. 6, but the arm of the tertads have not approached closely nor has any break occurred. The linin connections which are very evident were largely lost in reproduction.

Fig. 11. A nucleus in which the four chromatids of each tetrad are clearly visible. The double linin thread may be traced into the overlying chromatids of the right tetrad which bend back and down to become continuous with the two underlying chromatids which in turn are continuous with the second pair of strands of linin thread. The connections of the left tterad with these linin threads is so indistinct as not to be exactly traceable. The left tetrad is in such a position that three of its chromatids are visbile while the fourth may be traced by focusing down. The different chromatids are much connected by bridges.

Fig. 12. A pair of tetrads fully formed and lying in the maturation spindle, showing the characteristic angle between them.



GRIGGS on "Ascaris."